Friedrich-Schiller-Universität Jena
Faculty of Biology and Pharmacy

The Janus-faced role of secreted sphingomyelinase and its inhibition in host response

Dissertation
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Reviewers:

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I- Introduction

1. Sepsis: from Definition to Pathophysiology

Sepsis is a life threatening disease and the leading cause of mortality in intensive care units worldwide (1). With a continuous rise in incidence, sepsis is a major health concern (2) retaining a significant and tremendous burden on the health care system registering an average annual cost of 16.7 billion US dollars back in 2008 (3). Although sepsis has been reported for over 2000 years, standardized clinical definitions are relatively recent but have been established for the systemic inflammatory response syndrome (SIRS) as well as the continuum of the sepsis syndrome from sepsis to severe sepsis and septic shock (3, 4). Sepsis itself is defined as the host's innate and immunological response to an infection. An infectious etiology is required for the diagnosis of sepsis (5) and there has been a wide variety of identified infectious sources. Although bacteria remain the main source of infection with gram positive bacteria (namely *Staphylococcus aureus*) being the most commonly isolated in sepsis, fungal organisms (namely *Candida spp.*) have been increasingly reported (**Figure 1.1**) (3, 4).

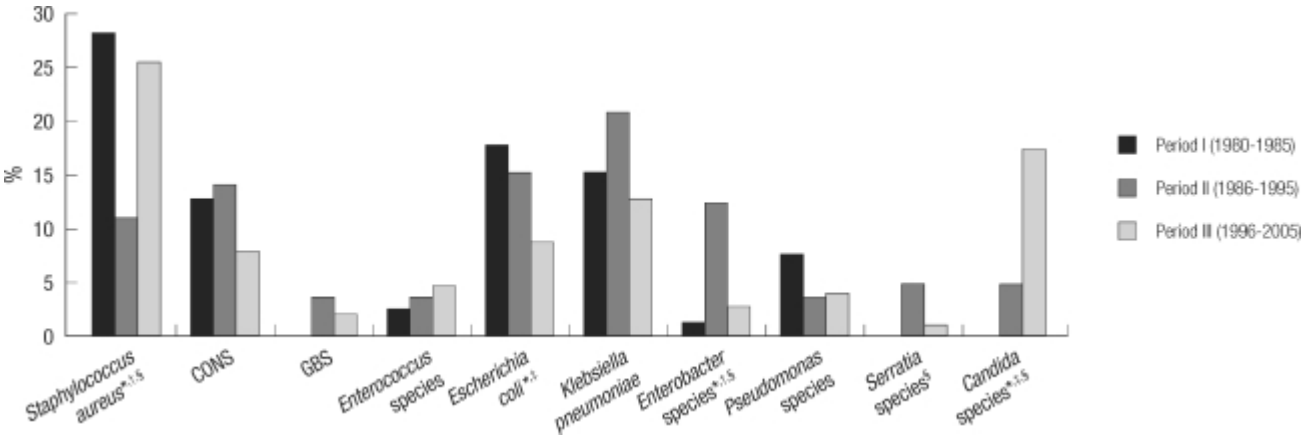


Figure 1.1: A representation of the most commonly isolated microorganisms in neonatal sepsis over three periods of time between the years 1980 and 2005. *Staphylococcus aureus* and *Klebsiella pneumoniae*, were the most commonly isolated gram(+) and gram(-) bacteria in sepsis between 1996 and 2005. *Candida spp.* infections have been on the rise since 1996. Abbreviations: CONS- coagulase negative *Staphylococci*, GBS- Group B *Streptococcus.* The Figure is taken from Shim GH. *et al.* (2011) (6).

To this day, the pathophysiology of sepsis is not completely understood but is known to involve complex inflammatory and physiological processes. Following extensive research, the understanding of sepsis developed from the introduction of "wound putrefaction" by Hippocrates, the implication of fever in septicemia by Ibn Sina and the identification of bacterial presence decades later by Louis Pasteur to Hugo Schottmüller laying the groundwork of modern sepsis definitions (1914) by describing the presence of an infection as a fundamental component of the disease. Yet, it was the theory put through by Lewis Thomas that polarized and completely altered the understanding of the pathophysiology of sepsis by introducing the theory that it is the host response that drives the development of the syndrome (7, 8). The present concept was introduced into daily clinical practice by Roger Bone and colleagues who defined sepsis as "a systemic inflammatory response syndrome (SIRS) that can occur during infection" (7, 9). We now know that the host defense responses are initiated during tissue damage and microbial infections by pattern recognition receptors (PRRs) expressed on immune cells which recognize pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) expressed on microorganisms and damaged tissues respectively, subsequently initiating the immune response. A full-scale systemic immune activation leads to an overstimulation of the immune cells resulting in an imbalanced and overwhelming cytokine storm with a variety of cytokines, chemokines and complement factors which cause an excessive collateral damage involving different cell types and remote organs (7, 10). Additionally, the pro-inflammatory response induces the expression of secondary mediators such as reactive oxygen species (ROS) and bioactive lipids that further amplify the immune response (7, 11). In fact, several biological systems and cell types play a role in sepsis thus resulting in severe dysregulation of the inflammatory network. It is a syndrome with great heterogeneity that is also affected by a variety of premorbid factors as well as age and gender (7, 12).

Sepsis can be complicated through the development of organ failure namely severe sepsis followed by the development of septic shock with which mortality is radically increased (2) (**Figure 1.2**). It is associated with a haemostatic imbalance together with hypotension and compromised microcirculation resulting in impaired organ

perfusion. In fact, the microcirculatory status and the disturbances in microcirculation are key elements in the development of septic shock (13, 14). The liver plays a pivotal role in sepsis where hepatocyte cross-talk controls most of the inflammatory and coagulation processes. A continuous increase in cellular stress could result in liver dysfunction through compromised circulation and perfusion leading to an enhanced procoagulant and inflammatory process and eventually multiple organ failure (15, 16).

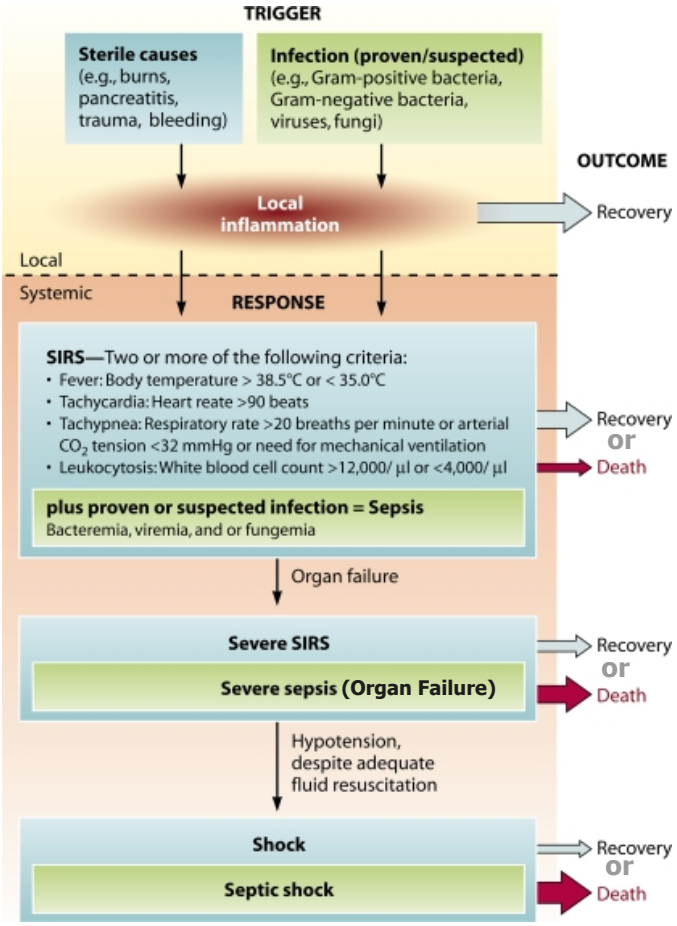


Figure 1.2: The disease continuum. Inflammation is the host's response to an infection or an insult. Normally, a local inflammatory response is usually resolved. However, if the inflammation becomes dysregulated, systemic activation of the innate immune system can occur. The complex clinical findings associated with this systemic activation are known as SIRS which is triggered by sterile inflammatory processes. SIRS presents with at least two of the following clinical findings: fever or hypothermia, tachycardia, tachypnea, or leucopoenia or leukocytosis. Sepsis is defined as a suspected or proven infection plus a SIRS. SIRS evolves to severe SIRS and sepsis to severe sepsis with the involvement of organ dysfunction (e.g., hypotension, oliguria, and thrombocytopenia). Severe sepsis is called septic shock when it is complicated by refractory hypotension. Analogously, severe SIRS (accompanied with organ dysfunction) can lead to shock. At each stage of the disease, recovery is

possible. However, the patient's survival chances decrease substantially in the later stages of the disease. The Figure is modified from Vanlaere I. *et al.* (2009) (17).

2. Inflammation-triggered cytokine release

Invading microorganisms trigger a cytokine mediated pro-inflammatory response resulting in the development of sepsis. This involves a combination of exaggerated inflammation and immune suppression. The latter, recently identified as compensatory anti-inflammatory response syndrome, is characterized by anti-inflammatory mechanisms which are observed during the development of sepsis.

Among the most widely addressed cytokines, TNF-α and IL-1 activate target cells which, once triggered, induce the generation of other cytokines (18). TNF-α dependent IL-6 is both a pro- and anti-inflammatory cytokine, which along with IL-8, IL-12, IL-10, interferon (INF)-γ and the granulocyte-colony stimulating factor (G-CSF), plays a pivotal role in the regulation of the host response (18). Additionally, IL-17 is a pro-inflammatory cytokine that has been recently implicated in the pathogenesis of sepsis through its ability to induce the generation of other inflammatory mediators such as IL-1b, IL-6 and TNF-α (19). Its inhibition in a murine sepsis model has resulted in the reduction of bacteremia and the systemic levels of cytokines associated with an improvement in survival (20). Other newly recognized cytokines include the macrophage migration inhibitory factor (MIF) and the pro-inflammatory cytokine HMGB-1 which, when inhibited, have revealed an improvement in survival and protection against septic shock (21-24). However, these cytokines are not the sole triggers or drivers of SIRS and sepsis. In fact, therapeutic approaches based on a complete blockage of the inflammatory response have failed with respect to reducing mortality which highlights the complexity of sepsis (25, 26).

3. Leukocyte recruitment during Host Response

During inflammation and infection, a variety of cells are recruited to the site of inflammation including leukocytes, fibroblasts and endothelial and epithelial cells (27). Leukocyte recruitment involves a multi-step event cascade (**Figure 1.3**). Primarily, leukocytes migrate out of the blood circulation through the blood vessel wall. This process is initiated by tethering followed by leukocyte-endothelium interaction which begins with the rolling of white blood cells across the endothelium.

This mechanism is initiated through the expression of P and E selectins by the activated endothelium. Additionally, leukocytes express L-selectins thus resulting in optimal leukocyte-endothelium interaction (28). Rolling is followed by sticking or firm adhesion on the endothelium which involves integrins such as β_2 and/or α_4. Leukocyte integrins are activated by G-protein coupled receptors that are triggered through the latter interaction between the leukocytes and the endothelium with the help of chemokines (29). Activated integrins in turn firmly bind to endothelium ligands such as ICAM-1 and VCAM-1 resulting in firm adhesion. This adhesion is strengthened by signaling of chemokine receptors (30, 31).

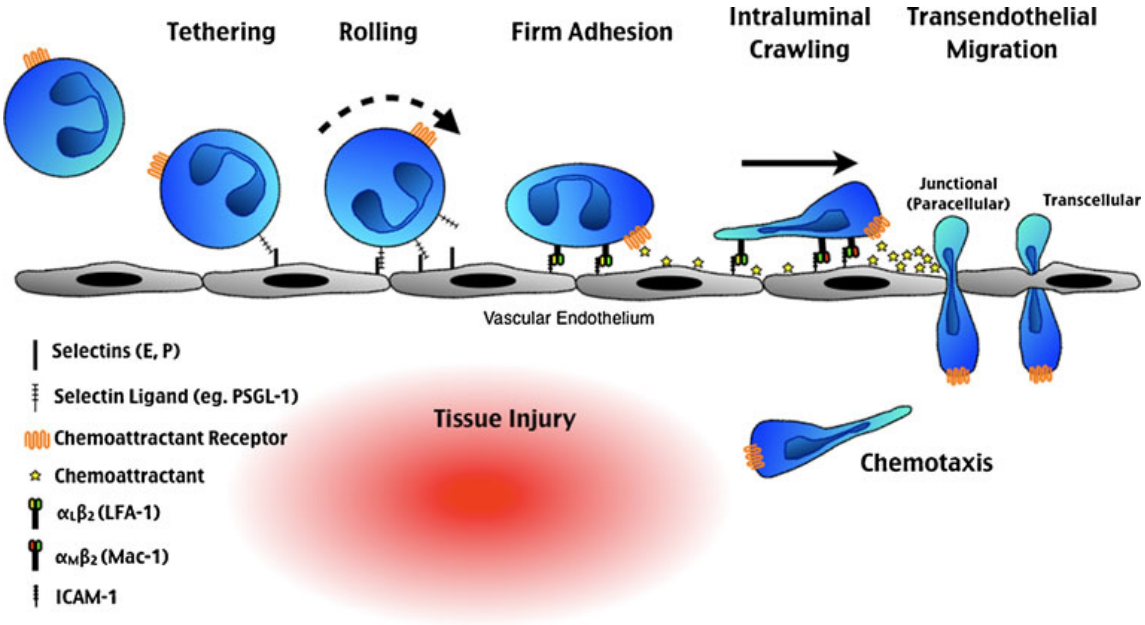


Figure 1.3: The classical neutrophil recruitment cascade. Initial tethering and rolling events are mediated by binding of endothelial selectins to neutrophil selectin-ligands. Subsequent engagement of chemokine receptors on rolling neutrophils activates integrins (LFA-1) leading to firm adhesion followed by crawling. Neutrophils then exit the microvasculature by transendothelial migration between endothelial cells or directly through endothelial cells. Once they reach the target tissues, neutrophils migrate to the site of infection guided by gradients of chemoattractants. The figure is taken from McDonald B. *et al*. (2011) (32).

Adhesion is followed by leukocyte crawling leading to emigration from the vessel into the tissue. Crawling is a process by which activated leukocytes move across the endothelium to the nearest endothelial junction, crucial for efficient emigration (33). Various proteins have been implicated with leukocyte emigration and include platelet endothelial cell adhesion molecule-1, CD99, junctional adhesion molecules and

vascular endothelial-cadherins (34). Following emigration into the tissues, leukocytes migrate to the site of infection guided by gradients of chemoattractants such as chemokines (primarily CXCR2 ligands for neutrophils), lipids (including LTB4, PAF) and complement anaphylotoxins (C5a and C3a) (32).

Although the inflammatory response as well as leukocyte recruitment are crucial for fighting the infection, these events, especially the acute response of neutrophils, play also a substantial role in the pathology of sepsis-associated organ dysfunction (35). Once activated, emigrated leukocytes are sequestered in the capillary networks of organs such as the liver promoting organ dysfunction (27, 35-37) through hepatocellular damage or vascular hypoperfusion (15).

4. Acid sphingomyelinase, Ceramide and Cellular stress response

Sphingomyelin is a ubiquitous sphingolipid found in the nuclear envelope and intranuclear sites. Its role in cell signaling has become a major research focus with respect to its primary product ceramide, an emerging bioactive lipid and secondary messenger (38, 39). The breakdown of sphingomyelin to ceramide occurs through several pathways including the extracellular, stress-induced activation of acid sphingomyelinase (aSMase) that breaks down membrane bound sphingomyelin thus releasing ceramide (**Figure 1.4**) (39, 40). Acid sphingomyelinase was previously considered a "house-keeper" basically known for its role in Niemann Pick disease. However, we now understand that aSMase is triggered by a variety of stress stimuli and receptors including CD95, tumor necrosis factor receptors (TNFR), Toll-like receptor 4 (TLR4), CD5 as well as bacterial and viral infections thus shifting the attention over the past decade to the role of this enzyme in regulating ceramide generation and to the function of ceramide during host response (41, 42). The availability of the complete loss of function model with aSMase ko animals shed more light on the crucial role that this enzyme plays. These ko animals appear to be protected against a variety of stress stimuli such as radiation triggered cell death owed to impaired ceramide generation (43, 44). The upregulation of secreted aSMase was originally reported in an endothelial cell culture following cytokine-triggered inflammation and was later reported in an animal model of endotoxemia (42). Acid sphingomyelinase has been purified from urine of patients with peritonitis

and was found to be elevated in these patients as compared to healthy controls (45). In 2005, plasma collected from critically ill patients revealed a continuous increase in levels of secreted aSMase with disease progression and severity from SIRS to sepsis, severe sepsis and multiple organ failure (46). With the increasing reports on the role of aSMase in response to infection, we now understand that once triggered, the enzyme is translocated to the outer leaflet of the plasma membrane resulting in a rapid and transient increase in ceramide generation which in turn accumulates forming ceramide-enriched membrane platforms also known as lipid rafts (**Figure 1.4**) (42, 47).

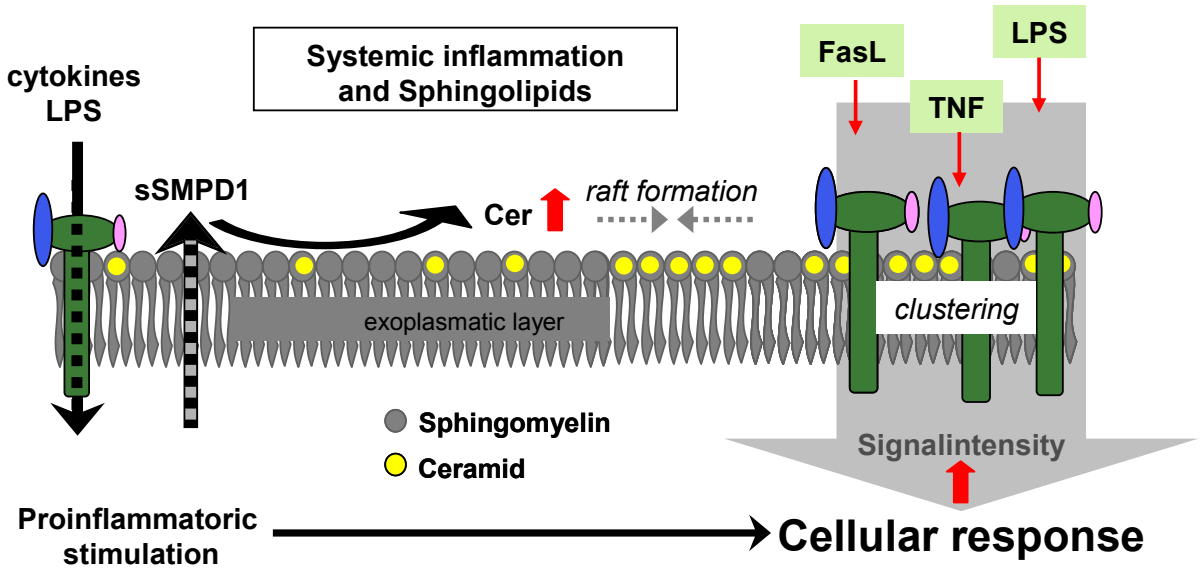


Figure 1.4: An exogenous stimulus triggers a pro-inflammatory stimulation resulting in the secretion of aSMase to the exoplasmatic layer. The enzyme breaks down sphingomyelin into ceramide which accumulates forming lipid rafts which develop into ceramide macrodomains. Receptors cluster and are activated in macrodomains resulting in an increase in signal intensity and triggering the cellular response. The Figure is taken from Hupe D., Dissertation, Jena University (2008).

Ceramide and lipid rafts regulate the immune system either directly by signaling events or indirectly by affecting pro-inflammatory cytokine release, crucial in host response to infection. It has been shown that ceramide-mediated signaling is expressed by a variety of cells of the immune system (48, 49).

Ceramide generation within rafts alters the biophysical properties of the membrane microdomains. In fact, ceramide tends to fuse small rafts forming ceramide-rich membrane platforms or macrodomains that serve as mediators for cell signaling.

These domains have been associated with clustering of receptor molecules, reorganization of signaling proteins and the exclusion of inhibitory signaling factors (47, 50). Once formed, they serve to cluster and signal CD95 and CD40 to name a few thus facilitating and amplifying signaling leading to cellular activation and ultimately apoptosis (50). Ceramide rich platforms recruit the TLR-4 complex which, when activated, plays a crucial role in the LPS response by macrophages (51). Also mediated by the raft modifications are the recruitment of FADD, caspase 5 and caspase 3 to aggregate CD95, crucial for apoptosis. Indeed, ceramide appears to be crucial for all three types of apoptosis; death receptors *i.e.* CD95, DR5 and TNF-triggered apoptosis, non-receptor stimuli such as heat shock, toxins, H_2O_2 and microorganisms and finally deprivation of cells from growth factors (47, 52).

Many receptors present outside rafts are also trapped in these macrodomains. The resulting high receptor density allows signal transmission to the cells. These signals could serve to recruit and/or exclude intracellular signaling proteins. Ceramide activates a variety of proteins that regulate cell transcription, proliferation and survival. Ion channels have also been identified as a target of ceramide enriched platforms which inhibit a potassium channel and a calcium channel both pivotal for the activation, differentiation, proliferation and regulation of apoptosis. However, it is still unclear how ceramide functions with respect to blocking these channels (47, 52).

Additionally, various microorganisms utilize lipid rafts during infection whether it is for the internalization of bacteria in cases of *Neisseria gonorrhea*, for attachment of the pathogen in *Pseudomonas aeruginosa* infections or for the uptake of viruses such as rhinoviruses (47).

5. Role of acid sphingomyelinase in Pathophysiology

In cancer, a disease characterized by uncontrollable proliferation und compromised apoptosis, levels of ceramide appear to be significantly diminished especially in human colon cancers, gliomas and ovarian cancer. This suggested a downregulation of aSMase with subsequent reduction in ceramide generation thus inhibiting the apoptotic pathway. The aSMase pathway has also been associated with various antioncogenic treatments with the enzyme retaining a crucial secondary role in the action of these drugs. aSMase has also been linked to angiogenesis where aSMase ko

animals appear to be resistant to radiation therapy thus suggesting a crucial role of the enzyme in triggering irradiation-induced apoptosis (43, 44).

Although aSMase appears to be beneficial in oncology, its role appears to be detrimental in chronic inflammation such as cardiovascular disease where a significant increase in the enzyme activity has been measured in patients with chronic heart failure (associated with cachexia and mortality) as well as in atherosclerosis where the enzyme is involved in the formation of atherosclerotic plaques (53). Diabetic patients, presenting with a higher risk of developing atherosclerosis, also registered elevated levels of plasma secreted aSMase. The enzyme has also been associated with insulin resistance through negative modulation of the insulin receptor substrate (IRS-1) by ceramide (43, 54-56).

Acid sphingomyelinase is crucial for maintaining a normal brain function as suggested by the neurodegenerative disorder in patients with Niemann Pick disease, yet altered levels of the enzyme have been associated with depression, Alzheimer's disease and ischemia. Higher aSMase activity and ceramide levels have been registered in patients with major depression as well as Alzheimer's disease. Additionally, in a mouse model of cerebral ischemia, wt animals revealed elevated aSMase activity as well as ceramide and cytokine levels that were not observed in aSMase ko mice which reflected a better outcome in the latter animals (43, 57).

A role of aSMase in pulmonary disease has been reported in emphysema and cystic fibrosis, to name a few. A registered elevation in ceramide levels has been reported in patients suffering from emphysema. According to Petrache *et al.*, ceramide retains a crucial role through the activation of receptors ultimately leading to alveolar cell apoptosis and alveolar destruction, crucial in the pathogenesis of emphysema. In cystic fibrosis, a role of aSMase with respect to bacterial spread and persistence has been elucidated. In fact, aSMase ko animals or those treated with a pharmacological inhibitor of the enzyme appear to be more resistance to *Pseudomonas aeruginosa* infections with a decrease in the baseline of pulmonary inflammation (43, 58).

As previously mentioned, aSMase and ceramide also play a complex and crucial role in infection with respect to microorganism internalization and survival yet these processes, especially in sepsis, are not entirely understood. However, aSMase activity is reportedly elevated in endotoxemia reaching a 2 to 2.5 fold increase (43, 59).

6. Inhibitors of the acid sphingomyelinase enzyme

Although potent and selective inhibitors of aSMase have been identified, they present with major limitations and disadvantages with respect to toxicity, stability and cell penetration (60). On the other hand, desipramine and imipramine, well-established drugs for the treatment of major depression, were later found to be functional inhibitors of acid sphingomyelinase (FIASMAs) and appeared to efficiently reduce the levels of aSMase with no cytotoxic side effects (60, 61). They function by detaching the aSMase enzyme from the inner lysosomal membranes resulting in its deactivation and degradation (**Figure 1.5**). It is therefore a non-specific inhibitor requiring high lysosmal concentrations which are reached by lysosomal trapping due to physicochemical properties.

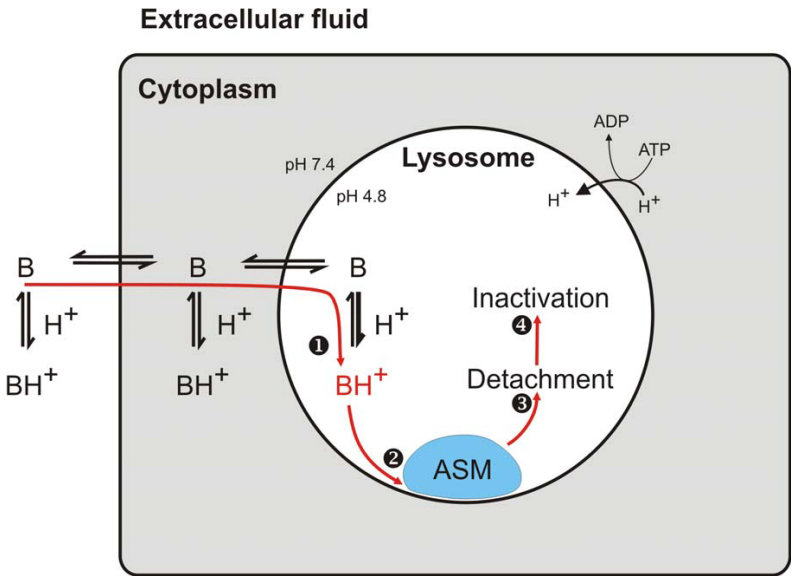


Figure 1.5: Drug-induced functional inhibition of aSMase is the result of lysosomal accumulation and detachment of aSMase from the inner lysosomal membrane. The figure shows a schematic model of how FIASMAs (functional inhibitors of acid sphingomyelinase) and other lipophilic weak bases cumulate intra-lysosomally, thereby functionally inhibiting aSMase. A low lysosomal pH is maintained by an ATP-driven proton pump. (1) Weak bases (B) cumulate in intracellular acidic compartments because the lysosomal membrane is much less permeable to the charged protonated bases (BH+) compared to the uncharged form. The enzyme aSMase is attached by electrostatic forces to the inner lysosomal membrane, thereby being protected against proteolysis. (2) High concentrations of the protonated bases disturb the binding of aSMase to the inner lysosomal membrane and result in detachment of aSMase (3) and subsequent inactivation (4), possibly involving proteolysis [89]. The figure is taken from Kornhuber J. *et al.* (2008) (62).

Most of the available FIASMAs possess appropriate ADME properties (Absorption, Distribution, Metabolism and Excretion). Although they have variable lyososmal uptake characteristics, desipramine is a FIASMA with a fast uptake kinetic as well as a moderate lysosomal accumulation. These inhibitors are also capable of extensively binding to the tissue due to their special physicochemical properties, *i.e.* weak basicity and high lipophilicity (63, 64). It is important to note however, that FIASMAs do not result in a complete inhibition of the enzyme but rather retain a residual aSMase activity thus avoiding the accumulation of sphingomyelin and the development of Niemann-Pick disease. Additionally, pharmacological inhibition of the enzyme is reversible as levels of aSMase return to control values three days following the drug withdrawal (57, 65).

As inhibitors of aSMase with a resulting attenuated receptor-induced apoptosis, FIASMAs present with a possible broad clinical application for the treatment of common diseases (66). Their anti-apoptotic and neuroprotective effects are promising for the treatment of neurological disorders such as Parkinson's disease, multiple sclerosis, brain stroke and ischemia (61, 67, 68). FIASMAs are also promising for the treatment of liver disease and anemia in Wilson's disease (69), inflammatory bowel disease (70), atherosclerosis and heart disease (43) as well as cystic fibrosis (65, 71). Ceramide plays a key role in acute and chronic lung disease which could be alleviated with the use of these aSMase inhibitors (58, 72). FIASMAs are used for treatment of major depression which has been associated with diminished aSMase activity (57). Preliminary results using the endotoxemia model also suggest a possible advantage for the use of FIASMAs in infection and sepsis (46, 73).

7. Aim of the Project

Here we elucidate the role of aSMase in the acute phase of sepsis using the peritonitis sepsis model. We aimed to define and establish the peritoneal contamination and infection (PCI) model with respect to reproducibility, reliability and transferability to the clinical setting and with respect to course by measuring various parameters of host response including systemic cytokines, bacterial burden and markers of organ (dys)function. Liver dysfunction and changes in microcirculation and leukocyte-endothelium interaction were additionally evaluated by intravital microscopy. A complete set of experiments was also performed comparing PCI to another commonly used animal model of systemic inflammation, the endotoxemia shock model.

To elucidate the role of aSMase in sepsis, I compared the complete loss of function model to wt littermates and wt animals pretreated with a pharmacological inhibitor, desipramine. I stratified the role of the enzyme in host response through the analysis of established parameters of host response such as bacterial burden, cytokines, leukocyte and platelet counts, markers of organ (dys)function and ROS release. I also stratified the leukocyte phenotype by intravital microscopy, gene expression and analysis of surface protein expression. Additionally, I analyzed the effect of aSMase activity and its role with respect to outcome by performing a survival analysis. I also investigated the possible beneficial role of the functional inhibitor desipramine in ameliorating the host response to sepsis addressing its possible use in the treatment of the host response.

II- Manuscripts

Manuscript 1: Gonnert *et al.* (2011). *Journal of Surgical Research.*

Characteristics of clinical sepsis reflected in a reliable and reproducible rodent sepsis model.

Summary:

For the purpose of mimicking the clinical situation of diffuse peritonitis in a reliable murine sepsis model, we aimed to establish a standardized and reproducible model, PCI or peritoneal contamination and infection to be utilized in the present project. This method has the advantages of simplicity and limited variability in outcome as compared to other commonly used sepsis models. Briefly, we collected human stool samples from healthy non-vegetarian donors. Following microbiological analysis and stool preparation and preservation, a standardized dilution of fecal slurry was injected intra-peritoneally (IP) to mimic diffuse peritonitis sepsis in humans. In order to characterize the model, we performed several experiments including the measurement of cytokines, blood counts, blood gas analyses and markers of organ (dys)function as well as several trials of survival analysis to confirm reproducibility. The summation of results clearly indicated an infectious focus and the development of diffuse peritonitis. Laboratory findings were very similar to the clinical course of patients with sepsis. Results support the standardization and reproducibility of the PCI method associated with various prototypical features of sepsis and multi-organ failure.

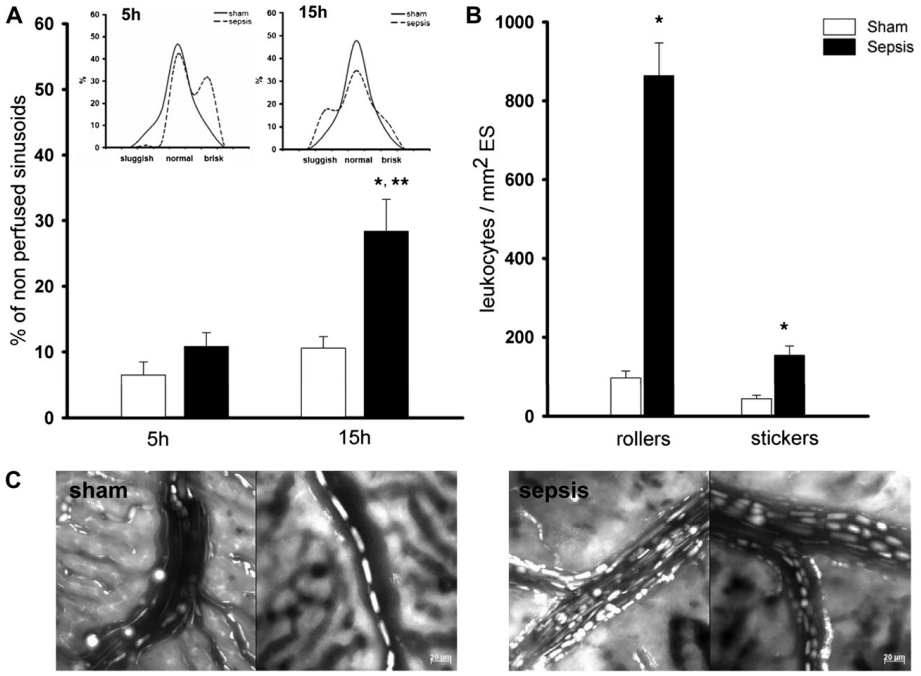
Reference: Gonnert FA, Recknagel P, Seidel M, **Jbeily N**, Dahlke K, Bockmeyer CL, Winning J, Lösche W, Claus RA, Bauer M. (2011). Characteristics of clinical sepsis reflected in a reliable and reproducible rodent sepsis model. *J Surg Res.* 170:e123-34.

Author's Contribution:

To complete the picture with respect to organ involvement during host response, I performed intravital microscopy of the liver using an epifluorescent microscope to evaluate changes in leukocyte-endothelium interaction, highlighted by rolling and sticking. I also evaluated changes in liver microcirculation and perfusion. Following the experiments, I analyzed the videos and images and assisted in preparing the related figures for the manuscript. I also assisted with the literature and discussion with respect to microbiology analysis and survival of microorganisms as well as revision of the manuscript before submission.

Signature ----------

Dr. Ralf Claus



Evaluation of hepatic microcirculation. Intravital microscopy (IVM) of the liver was performed in four animals per group. (A) Microcirculation was analyzed at 5 and 15 h post-insult. The average percentage of (non-)perfused sinusoids is indicated. The inset depicts the quality of flow that was determined in perfused sinusoids at 5 and 15 h. (B) Leukocyte-endothelium interaction was analyzed in post-sinusoidal venules 15 h post-insult. Adherent leukocytes that did move or detach from the endothelium prior to a period of 30-s were defined as 'rollers'. Those that adhered to the endothelial wall for longer were classified as 'stickers'. The number of rollers and stickers were calculated per mm2 of endothelial surface (ES). (C) Rolling and sticking of *in-vivo* stained leukocytes demonstrated in representative overlays of 30-second video sequences. Data is given as mean ± SEM, *<0.05 versus sham, **<0.05 versus sepsis 5 hours.

Manuscript 2: Recknagel *et al.* (2013) *Liver International.*

Mechanisms and functional consequences of liver failure substantially differ between endotoxemia and fecal peritonitis in rats.

Summary:

The endotoxemia shock model is frequently used to describe and understand the pathophysiology and mechanisms of the host response to sepsis. As part of establishing PCI as an efficient yet more clinically relevant model in mimicking the human situation with respect to sepsis and severe sepsis, we compared physiological and inflammatory changes in both polymicrobial peritonitis and endotoxemia. Both models induced the host response but interestingly so in a different way. Although both models resulted in a similar morbidity, the PCI model induced a more visible hepatic dysfunction with a significant increase in markers of cholestasis (serum bilirubin) as seen in clinical practice. Unlike PCI, LPS injection did not show signs of severe peritonitis but rather cell toxicity and death resulting from endotoxemia and shock. Our results display PCI as a reliable and clinically relevant sepsis model resulting in a more adaptive cellular response that is more physiologically and clinically relevant than endotoxemia.

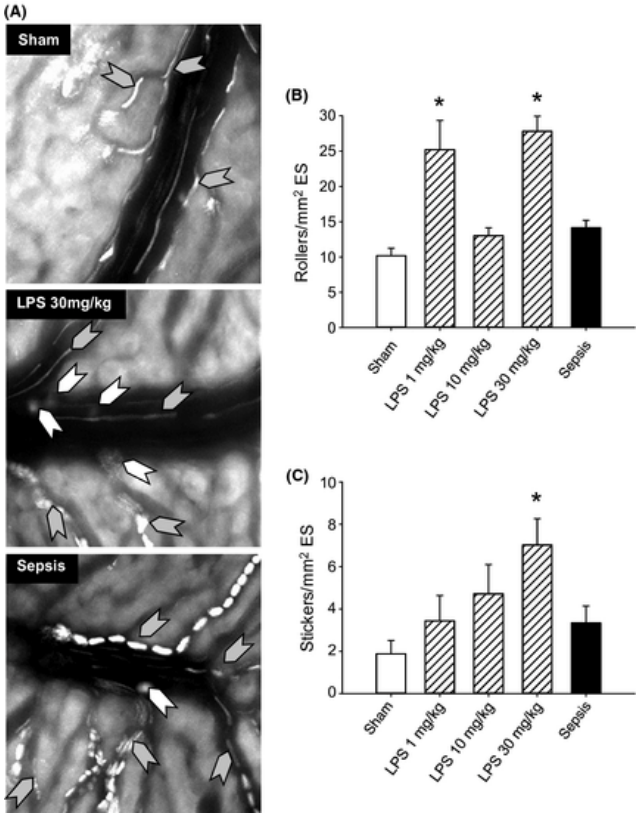
Reference: Recknagel P, Gonnert FA, Halilbasic E, Gajda M, **Jbeily N**, Lupp A, Rubio I, Claus RA, Kortgen A, Trauner M, Singer M, Bauer M. (2013). Mechanisms and functional consequences of liver failure substantially differ between endotoxemia and fecal peritonitis in rats. *Liver Int.* 33:283-93.

Author's Contribution:

To observe changes in leukocyte-endothelium interaction occurring in the liver, the addition of intravital microscopy was a valuable tool with which to assess these changes in post-sinusoidal venules and sinusoids comparing both models. As such, I performed intravital microscopy experiments of the liver using an epifluorescent microscope. At the end of the experiments, I analyzed the videos and images to generate the data which was represented in box plots and I prepared representative illustrations reflecting the results. I also assisted in animals handling and surgery. Finally, I assisted in the revision of the manuscript before submission.

Signature:----------

Dr. Ralf Claus



Leukocyte recruitment. Leukocyte-endothelium interaction in post-sinusoidal venules was analysed *in-vivo* by intravital microscopy 5 h' post-insult. Indicated are the (B) 'roller' count and (C) 'sticker' count per mm^2 endothelial surface (per 100 leucocytes; *<0.05 vs. sham). (A) depicts representative overlays of 30 sec movies of post-sinusoidal venules with corresponding sinusoids taken 5 h post-insult in sham-, PCI- and LPS (30 mg/kg BW)-treated animals respectively (original magnification 400×, n = 3 animals per group, five ROI per animal). White arrows indicate leucocytes that moved or detached from the endothelium within 30 s ('rollers'), grey arrows indicate leucocytes that adhered to the endothelial wall for >30 s ('stickers').

Manuscript 3: Jbeily *et al.* (2013). *Journal of Lipid Research*

Hyperresponsiveness of mice deficient in plasma-secreted sphingomyelinase reveals its pivotal role in early phase of host response.

Summary:

To investigate the role of aSMase in sepsis, we performed a set of experiments with the loss of function model (aSMase ko animals) compared to wt littermates using the PCI sepsis model. Since the increase in aSMase activity and the subsequent rapid and transient ceramide generation has been associated with an unfavorable outcome, we expected to observe an improvement in outcome (survival) with aSMase ko animals subjected to sepsis. Primarily, analysis of bacterial burden in different organs revealed a significantly elevated CFU in both wt and ko animals. Strikingly however, both genotypes presented with a similar survival (20% after 72 hours) but our data strongly suggested a different pathway between the two genotypes. In wt animals, the insult resulted in an increase in aSMase activity leading to rapid and transient formation of ceramide resulting in detrimental effects in the late phase of sepsis with subsequent high mortality. Although we expected a better result with respect to survival and overall outcome with the abrogation of aSMase activity, we rather highlighted a dual role of the enzyme with its beneficial role in the early phase of sepsis with respect to bacterial elimination. Therefore, the complete abrogation of aSMase in the ko animals proved to be detrimental resulting in a hyperresponsive state and an outcome similar to wt littermates through different pathways.

Reference: Jbeily N, Suckert I, Gonnert FA, Acht B, Bockmeyer CL, Grossmann SD, Blaess MF, Lueth A, Deigner HP, Bauer M, Claus RA (2013). Hyperresponsiveness of mice deficient in plasma-secreted sphingomyelinase reveals its pivotal role in early phase of host response. *J Lipid Res.,* 54:410-24.

Author's Contribution:

As the first author in this manuscript, I performed the experiments with the exception of the determination of phagocytotic activity, cytokine profiles (Iris Suckert), ROS generation (I supervised Sascha Grossmann) and gene expression (microarray). The experiments I performed included animals handling and surgery, sepsis induction, determination of bacterial burden (with the assistance of Iris Suckert), survival analysis, blood analysis for complete blood counts, leukocyte subpopulations and measurement of markers of organ (dys)function, histology studies for assessment of granulocyte migration (with the assistance of Iris Suckert), expression of surface markers of leukocytes by flow cytometry (while supervising Sascha Grossmann), measurement of aSMase activity in the plasma, isolation and preanalytics of leukocytes for ceramide measurements (ceramide measurements were performed by Anja Lüth in Potsdam) and intravital microscopy of the liver. I also assisted in real-time PCR experiments.

Finally, I generated all figures and prepared the manuscript. The final draft was revised by Dr. Ralf Claus and proofed by all co-authors.

Signature:----------

Dr. Ralf Claus

Manuscript 4: Jbeily *et al.* (2013). *Disease Models and Mechanisms*

Identification of a distinctive Leukocyte-Phenotype following pharmacological inhibition of aSMase during Host Response

Summary:

Sepsis is characterized by an overwhelming host response with limited treatment options. The results obtained with the use of the ko model urged us to apply a pharmacological inhibition of the enzyme using desipramine which does not completely abrogate the function of the aSMase enzyme and has shown to be promising in treatment of common diseases. We addressed the potential of desipramine, a functional inhibitor of aSMase, for regulating the host response. Indeed, we identified a distinctive leukocyte phenotype in animals pretreated with desipramine compared to vehicle-treated animals. The former animals presented with different gene expression profiles (1) resulting in alleviated cytokine levels in the late stage of sepsis and (2) abrogating the increase in ROS production in the early phase. The use of this inhibitor also resulted in an altered surface protein expression thus revealing a missing increase in leukocyte-endothelium interaction in the liver following sepsis induction. As a consequence, we observed alleviated liver dysfunction as measured by laboratory markers of organ dysfunction and an improved survival with desipramine pretreatment (42%) as compared to vehicle (20%).

Reference: Nayla Jbeily, Sascha D. Grossmann, Iris Suckert, Falk A. Gonnert, Tobias Ludwig, Anja Lueth, Burkhart Kleuser, Jürgen Rödel, Michael Bauer, and Ralf A. Claus (2013). Identification of a distinctive Leukocyte-Phenotype following pharmacological inhibition of aSMase during Host Response (**Submitted for review** in *Dis Model Mech.*)

Author's Contribution:

As the first author of the manuscript, I performed the experiments with the exception of the determination of phagocytosis activity, cytokine profiles (Iris Suckert) and ROS generation (I supervised Sascha Grossmann). The experiments included sepsis induction (PCI) and treatment of animals with desipramine for pharmacological inhibition of aSMase, survival analysis, determination of bacterial burden (while supervising Tobias Ludwig), blood analysis with complete blood counts and analysis of leukocyte populations, measurements of markers of organ (dys)function, histology studies for the assessment of granulocyte migration, surface protein expression on leukocytes by flow cytometry, measurement of aSMase activity in the plasma, isolation of leukocytes and preanalytics for ceramide measurements (performed by Anja Lüth), gene expression by qPCR (while assisting and supervising Sascha Grossmann) and intravital microscopy.

Finally, I generated all figures and prepared the manuscript. The final draft was revised by Dr. Ralf Claus and proofed by all co-authors.

Signature:----------

Dr. Ralf Claus

Manuscript 5: Jbeily *et al.*

Comparison of Carboxyfluorescein diacetate succinmidyl ester (CFSE) and Rhodamine 6G for *in-vivo* labeling of leukocytes [In Preparation].

Summary:

Intravital microscopy is an excellent tool for monitoring changes in the liver, an organ retaining a crucial role during the development of sepsis. Although Rhodamine 6G is currently the most commonly used dye for monitoring changes in leukocyte-endothelium interaction and microcirculation, we compare it to CFDA-SE with respect to efficiency of leukocyte labeling in intravital microscopy. The latter dye shows more specificity in labeling thus resulting in clearer imaging which is challenging with the use of Rhodamine 6G (absorbed by the background). Therefore, we propose the use of CFDA-SE, a better choice for leukocyte labeling in intravital microcopy.

Author's Contribution:

As a first author, I performed the experiments: (1) intravital microscopy of the liver using an epifluorescent microscope. Following animal preparation and catheterization, I performed imaging using the two dyes followed by video image analysis. I later analyzed the videos for leukocyte-endothelium interaction and prepared the graphs and representative images for the manuscript. (2) I also performed flow cytometry experiments to assess the ability of each dye to label different leukocyte subpopulations.

Finally, I generated all figures and prepared the first draft.

Signature: ----------

Dr. Ralf Claus

III- Discussion

1. The Burden of the Disease

As a research group focusing on and addressing a wide spectrum of the sepsis continuum, our combined efforts are to try to understand its complexity. Over the past few decades, researchers around the world have witnessed great inventions and developments in modern medicine that improved the quality of health care and extended the average life span. Yet, despite huge efforts, we still find ambiguities in several aspects of the medical field that require our attention (74-76). Although science, research and medicine have given us great insight into the complexity of sepsis, the worldwide incidence, morbidity and mortality rates due to sepsis are still on the rise and the treatment has become more challenging (77-79). Recent reports have registered a 9% increase in sepsis-related mortality rate per year in the US since the year 2000 (77). A 28.6% mortality rate was reported in the US following the evaluation of six million hospital records in seven states and 751,000 cases of sepsis (80). The average mortality rate due to sepsis in Brazil is stated to be around 29% (81). Mortality due to sepsis increases with the complication of severe sepsis where the UK for example recently reported a 35% mortality rate (82). In fact, with sepsis claiming the life of 20,000 people per day worldwide and with 1.8 million cases reported annually, the European Union alone has reported 90.4 cases of severe sepsis per 100,000 population (82), and sepsis research is therefore on a drive.

Today, there is a huge effort built into research to address the challenges of sepsis in the hope of compiling a more complete picture of the pathophysiology of the host response to infection thereby evolving more suitable, efficient and successful therapeutic options. Although *in-vitro* experiments with tissue cultures have been of great value, they are limited with respect to clinical relevance and transferability (83). For this purpose, various animal models of sepsis have been developed with the goal of mimicking the human condition thus giving a more in-depth insight into the clinical situation. Established animal models include intra-peritoneal (IP) injection of LPS or bacterial dilutions and cecal ligation and puncture (CLP), both of which present with advantages as well as limitations (84).

2. Added value of the PCI model for sepsis research

As part of the present project, we aimed to standardize and characterize the PCI model to be utilized by our research group as well as in the present experimental setup addressing the role of aSMase in sepsis. This model is clinically relevant as sepsis is triggered by a polymicrobial intra-abdominal infection that closely reflects the clinical situation observed in 50% of patients admitted to the ICU due to sepsis, severe sepsis and septic shock (85, 86). It is a simple method comprising an IP injection of a standardized and characterized dilution of fecal slurry. Microbiological analysis was performed following the collection of human faeces from healthy non-vegetarian donors which revealed the presence of a mixture of aerobic and anaerobic microorganisms that are frequently isolated in intra-abdominal infections such as *E. coli* and *Bacteroids* (87). An intra-peritoneal injection of a standardized stool suspension results in a diffuse peritonitis characterized by an overwhelming infection and paralleled with a systemic inflammatory response thus resulting in the clinical manifestations of sepsis. We measured a significant increase in bacterial burden following sepsis induction in organs and blood indicating a wide spread of the infectious focus accompanied by a significant increase in systemic cytokine levels. We were also able to observe involvement of remote organs as demonstrated by the increase in various laboratory markers of organ (dys)function following PCI. For example, we observed an early increase in markers of hepatocellular (dys)function and cholestasis as well as microcirculatory changes and enhanced leukocyte-endothelium interaction reflecting the development of liver dysfunction (87).

As reproducibility is a critical requirement for the reliability of a sepsis model, we repeated survival experiments over a period of 16 months using a frozen stool batch which confirmed the reproducibility of PCI (87).

3. Translational aspects of the PCI model

The measured parameters of host response and the reproduced experiments proved PCI as a reliable and clinically relevant model mimicking the clinical condition of diffuse peritonitis. Recently, a paper by Seok J. *et al.* addressed the reliability of different murine models of inflammation, including CLP- and endotoxemia, by comparing the genomic response in these models with the corresponding human

inflammatory diseases. With respect to the endotoxemia shock model, the authors concluded that this model is unreliable and poorly mimics the clinical situation (88). Unlike PCI, the endotoxemia model is based on the injection of LPS, a prototypic pro-inflammatory mediator from gram negative bacteria, which leads to a strong yet short lived immune response (89). In fact and as part of our objective to characterize and standardize the PCI model, we performed a set of experiments comparing polymicrobial intra-abdominal infections (fulfilled by PCI) to the endotoxemia shock model (LPS injection). We were able to demonstrate that where the PCI model induces a more visible hepatic dysfunction with a significant increase in markers of cholestasis (serum bilirubin) as seen in clinical practice, the LPS model did not show any signs of severe peritonitis but rather cell toxicity and death resulting from endotoxemia and shock (90). Also addressing other models such as CLP, Seok J. *et al.* concluded that all animal models are unreliable and poorly mimic the clinical situation. On the other hand, Lambeck *et al.* underlined the translational aspects of PCI through a comparison of gene expression in blood samples collected from murine animals following PCI and those collected from the pediatric ICU. The authors found significantly overlapping data in differentially expressed genes and could validate the reproducibility of PCI (91). Therefore, it is safe to suggest that the general conclusion reached by Seok J. *et al.* is not completely reliable and was unfairly generalized with respect to all animal sepsis models without sufficient supporting scientific grounds.

The widely and commonly used CLP sepsis model is one that is rather characterized by abscess formation which could contain the infection thus presenting with low-grade inflammation as opposed to diffuse peritonitis with subsequent sepsis (87, 92). Additionally, a constant leakage of faecal content cannot be guaranteed and the CLP procedure presents with limitations with respect to the standardization of the number of punctures as well as the manual extrusion of cecal contents (84, 93), namely operator inconsistency. This pitfall is resolved with the PCI model that does not require surgery. Moreover, the simple intra-peritoneal injection of the standardized stool dilution results in low variability (87). It could however be interesting and of great value to perform an additional set of experiments comparing the CLP and PCI models.

4. Long-term sequelae of sepsis

As shown by a recent report from our group, the PCI sepsis model also has long-term sequelae for surviving animals. In the post-acute phase, i.e. 28 days after sepsis induction, animals presented with abscess formation, liver fibrosis, elevated leukocyte counts and markers of liver (dys)function (94). Preliminary results of our group also include positive blood and tissue cultures 28 days following the septic insult with the use of antibiotics (for three days) which strengthens the transferability of this model This is in line with Otto *et al.* reported positive blood cultures in septic patients following diagnosis and antibiotic administration (**Figure 6**) with *Candida spp.* increasingly reported (30%) in the later phase of sepsis (95). Therefore the model seems to adequately reflect the clinical situation with respect to long-term sequelae of sepsis and as such, further research using this model is suggested for an in-depth understanding of the mechanisms in the post-acute phase of sepsis.

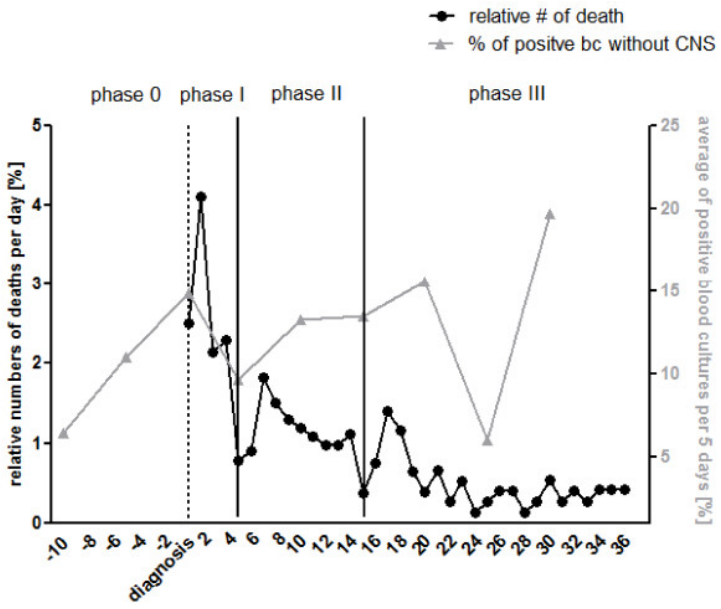


Figure 3.1: Distribution of non survivors and positive blood cultures during sepsis. Relative numbers of deaths per day from 999 patients with severe sepsis or septic shock according to ACCP/SCCM criteria are shown from the day of onset/diagnosis until observation Day 36. Three phases were defined, characterized by the nadir at Day 5 and Day 15. Also, the average rates of positive blood cultures without CNS in a five-day period with respect to sampling times are shown. Abbreviations: bc, blood cultures; CNS, Coagulase negative staphylococci; #, numbers; %, relative number. The Figure is taken from Otto GP. *et al.* (2011) (95).

5. Regulation of the Host Response by acid sphingomyelinase

As a key modulator of cell signaling, the roles of ceramide and aSMase in the pathology of common diseases have become a major research focus. Levels of aSMase were shown to significantly increase with disease progression from SIRS to severe sepsis and shock (46). Additionally, an upregulation in levels of secreted aSMase has been reported in chronic inflammation such as chronic heart failure (53). We therefore used the PCI model to address and characterize the role of aSMase in sepsis. This was possible with the availability of the loss of function model which allowed us to elucidate the role of aSMase in sepsis by comparing ko animals to wt littermates. This animal model used in the present study is a well characterized, highly reproducible and reliable animal model. It is commonly used to study the role of aSMase in a variety of common diseases such as cystic fibrosis (47, 48, 96). Primarily, the differences between the two genotypes were elucidated by the measurement of aSMase activity in the plasma and in leukocyte lysates as well as the levels of ceramide in circulating leukocytes at baseline as well as early sepsis. Following sepsis induction, the immune system is activated through a variety of stimuli and events, corner stones to for the development of sepsis. The presence of microorganisms creating an infectious focus results in the activation of the aSMase enzyme leading to the generation of ceramide from sphingomyelin (46). Indeed we measured a significant, sepsis-triggered increase in secreted aSMase in the plasma of wt animals also paralleled with a significant decrease in aSMase activity in a leukocyte lysate. As expected, aSMase activity in ko animals was negligible at baseline as well as following sepsis induction. This method is based on the hydrolysis of fluorescently labeled sphingomyelin which presents with sensitivity to light and therefore could, under false conditions, affect the results. This was however averted by the use of best-practice methods and by performing the experiment and subsequent analysis in the dark. Also, it is important to note that the results of aSMase activity measurements using fluorescently labeled sphingomyelin were similar to the use of radioactive-labeled sphingomyelin (46, 97). Additionally, maintaining a pH of 5.5 allowed the safeguarding of the enzyme and its products. Moreover, the levels of the intermediate product ceramide in circulating leukocytes were determined by an additional method independently (Mass spectroscopy)

through a collaboration with experts in the field. However, ceramide levels at baseline were significantly higher in ko animals compared to wt littermates. Resulting from an unknown compensatory mechanism (98), these ceramide levels at baseline (as well as after sepsis induction) are sufficient to maintain a normal function at a healthy state but not under septic conditions. The missing increase of ceramide in ko animals after sepsis induction outlines the pivotal role of this enzyme in the rapid and transient formation of ceramide.

Measuring the ceramide levels in circulating leukocytes required the isolation of these leukocytes from whole blood prior to analysis. This presented with challenges and limitations. The isolation procedure could *per se* result in the activation of the cells thus altering ceramide levels and resulting in false positive results. To overcome this challenge, we used the highly effective agent diisopropylfluorophosphate (DFP) which acts as a broad spectrum enzyme inhibitor preventing the activation of hydrolyzing enzymes during the isolation process. Another limitation was the loss of leukocytes due to the isolation procedure. In fact, cells might be lost or destroyed in the process of isolation. The analysis method however required at least one million cells per samples which urged us to pool leukocytes from several animals per group to achieve this goal. Therefore animal to animal deviations could not be observed in this experiment.

As the measured increase in aSMase activity in wt animals triggers a cellular stress response in the early phase which, in an overwhelming fashion, could act as a trigger of tissue damage in the late phase of the host response (73), we expected an improvement in outcome in ko animals due to the absence of continuous, rapid and transient ceramide generation. Surprisingly, survival in ko animals was similar to wt animals (20%). In subsequent experiments analyzing various parameters of the host response, we identified a different pathway in the ko animals that lead to a similar survival but highlighted a dual role of aSMase in sepsis. In wt animals, a complete set of data, with the continuous increase in systemic cytokines, ROS generation, leukocyte-endothelium interaction and markers of organ (dys)function, along with the measured increase in aSMase activity and ceramide levels following sepsis induction, led us to speculate that this increase could be the course or the consequence resulting in tissue damage, the development of organ failure and high

mortality in the late phase of host response. Thus, the increase in enzyme activity, leading to rapid and transient ceramide formation, appears to have a detrimental outcome. Surprisingly, the complete loss of aSMase triggered a hyperresponsive state ultimately implicating a pivotal role of the enzyme in the early phase of host response to sepsis. The completely abrogated aSMase activity resulted in a high mortality similar to wt, only through a different pathway. This was primarily highlighted with the analysis of the bacterial burden in blood and tissue where ko animals registered a significantly higher CFU in blood and liver following the septic insult as compared to wt. In fact, ceramide, produced actively by secreted aSMase, plays a pivotal role in elimination of microorganisms through the activation of the innate immune response (99). The absence of the enzyme, according to Ching *et al.*, left murine animals more susceptible to Sindbis virus-induced fatal encephalomyelitis (100). This phenomenon has also been observed in aSMase ko animals subjected to acute pulmonary infection with *Pseudomonas aeruginosa*. These animals, unlike wt, were unable to successfully eliminate the microorganisms from the lung and ultimately succumbed to sepsis a few days later (96). Therefore, the present data obtained with the analysis of the bacterial burden suggested that the aSMase enzyme retains a pivotal role in the early phase of host response with respect to bacterial elimination.

Although aSMase ko animals registered a significantly higher bacterial burden, the phagocytotic ability was intact as ko animals even registered a more pronounced increase in phagocytotic activity following the septic insult as compared to wt. According to Utermohlen *et al.,* the absence of the aSMase enzyme does not affect the uptake of the bacteria by phagocytes but rather results in a defective intracellular elimination (101). The overwhelming infection triggered a more pronounced response by leukocytes leading to a more significant increase in phagocytosis that was however inefficient. The present data suggested the inability of granulocytes to eradicate phagocytosed microorganisms possibly due to impairment in effector mechanisms (101).

The high bacterial burden in turn triggered an earlier and more pronounced inflammatory response in ko animals as compared to wt. The low molecular weight proteins cyto- and chemokines influence a broad range of cellular functions and have

been mainly classified into pro- or anti-inflammatory cytokines. Once released, they rapidly and transiently bind to specific receptor molecules triggering a cellular response in a time- and tissue restricted manner (102). In the present study, the absence of aSMase and the overwhelming bacterial burden had an effect on cytokine release where levels of TNF-α and IL-6 peaked earlier in ko animals, only six hours following the septic insult. On the other hand, the increase in aSMase activity resulted in an increased expression of TNF-α and IL-6 in an *in-vitro* model of cellular stress response, but the cytokine response was abrogated in both pharmacological inhibition and knock-down models (103).

The generation of ROS has been associated with ceramide-dependent TNF-α release (104), a fact that did not hinder ROS production by aSMase ko animals in the present study. This could however be explained by the 2-fold increase in levels of TNF-α measured in the latter animals which compensated for the impotence in rapid ceramide generation thus yielding a similar ROS release in the two genotypes during sepsis. Therefore, the hyperresponsive state in ko animals, triggering a pronounced cytokine release, also resulted in a significant increase in ROS production affecting the outcome.

The cytokine storm was paralleled with an upregulation in transcripts, encoding for cyto- and chemokines, in circulating leukocytes of both wt and ko animals. Six hours following the septic insult, in ko animals, we found an increase in the expression of *Tnfa* transcripts in whole tissue homogenates of the liver reflecting the elevated levels of systemic TNF-α. However, the expression of *Tnfa* in lung tissue remained unchanged. A difference between the two organs was also observed in the analysis of the bacterial burden with the liver registering an overall higher bacterial burden. This in turn triggered a more pronounced inflammatory response by the liver which retains a crucial role in host response that, together with its larger organ mass, the overwhelming bacterial burden and the pronounced cytokines levels in blood, suggested a different response pattern during host response (105) explaining a variable pathway in the expression pattern in this organ.

We also found a diminished expression of cathepsin G (*Ctsg*) in aSMase ko animals as compared to wt. Besides regulating phagocytosis, degranulation and ROS release, this serine protease has a role in the killing and digestion of engulfed microorganisms

(106, 107). Therefore, its diminished expression in our ko animals could have been a factor in the resulting overwhelming infection due to the hindered ability of leukocytes to eliminate phagocytised bacteria.

Although the absence of aSMase in ko animals had adverse effects with respect to triggering a hyperresponsive state, it also presented with a possible protective role with respect to the altered expression of surface proteins (CD49d, CD62L and CD11b) and the associated absence in leukocyte activation. The wt animals on the other hand, presented with a leukocyte profile that is associated with an unfavorable outcome. Leukocyte activation and recruitment in the liver could trigger macro- and microcirculatory changes as well as leukocyte-endothelium interaction. An increase in hepatic leukocyte-endothelium interaction, through leukocyte rolling and sticking, leads to leukocytes sequestration and the development of hepatocellular damage through continuous local release of pro-inflammatory cytokines eventually resulting in hepatic dysfunction (87, 108, 109). The increase in aSMase activity in wt animals, followed by rapid generation of ceramide and lipid rafts, allows the recruitment of surface proteins such as CD11b (110). The expression of surface protein CD11b together with CD49d and CD62L are essential for the rolling and sticking phenomenon (111, 112) which was observed solely in wt animals. Due to the downregulation of CD49d and the missing upregulation in expression of CD11b and CD62L on circulating leukocytes of ko animals, no increase in leukocyte-endothelium interaction was observed.

Changes in leukocyte-endothelium interaction were analyzed by intravital microscopy, a valuable investigative tool to elucidate changes in the liver during sepsis. However, it presents with challenges and limitations such as subjectivity in data analysis. This limitation was overcome by blinded analysis of the generated videos and images (no reference to the group) by two independent experienced scientists (Falk Gonnert and myself) thus limiting the subjectivity in the analysis process. Additionally, although Rhodamine 6G is the most commonly used dye in intravital microscopy (113), it presents with the limitation of being rapidly absorbed by the liver resulting in a bright background that is challenging with respect to data analysis. For this purpose, we chose to use CFDA-SE in the experimental setup as it

appeared to be more specific with respect to labeling of leukocytes thus presenting with clearer images.

Comparing the two genotypes, a variety of measured parameters suggested a different pathway that however resulted in a similar, unfavorable outcome with only 20% survival. The study of these two genotypes allowed us to elucidate a dual role of secreted aSMase in sepsis highlighting the crucial role this enzyme plays in the early phase of sepsis and the first line of defense against invading microorganisms. The abrogation of aSMase led to an overwhelming infection triggering a more pronounced inflammatory response and the progression of a generalized host response. On the other hand, an increase in activity with continuous ceramide generation appears to be detrimental in the late phase of host response as observed in wt animals.

However, although the ko model presented with valuable scientific data, the loss of function model is limited with respect to transferability. The complete loss of function results in the development of Niemann-Pick disease which limits the usage of these animals to a maximum age of 10 weeks. As age is a factor known to affect the outcome in sepsis patients, with a more unfavorable outcome with older age, the use of older animals is of great value to address the role of aSMase with respect to age. However, this is not possible with the loss of function model. We can however overcome this limitation with the use of pharmacological inhibitors of aSMase which would allow us to bridge the gap between the experimental setup and the clinical situation. As pharmacological inhibition with desipramine could not result in the development of Niemann-Pick disease (61), older mice could be used for analysis of a possible variability with respect to outcome and is yet to be addressed but presents as an interesting perspective of the present study. In addition, due to its advantage with respect to translational research, desipramine is also a FDA approved drug that is already available and used to treat cases of clinical depression. This further encourages the elucidation of its possible benefits for the treatment of sepsis. However, for comparability reasons, we used young animals for pretreatment with desipramine similar to the loss of function model and wt littermates.

Additionally, with respect to enzyme activity, establishing and utilizing a heterozygote model is another interesting perspective in comparison with the established ko and pharmacological inhibition models.

6. Pharmacological inhibition of the enzyme – Potential therapeutic use

Since it became evident to us that aSMase is crucial in the early phase of host response, it is important to note that the use of pharmacological inhibitors such as desipramine does not result in a complete inhibition of the enzyme (61). Residual activity therefore prevents the development of the hyperresponsive state observed in ko animals and the alleviated levels of aSMase could protect against the detrimental effects of the enzyme in the late phase. Indeed, desipramine pretreatment alleviated the increase in aSMase secretion following PCI with significantly lower levels of aSMase activity in plasma as compared to wt following the septic insult. As such, aSMase activity in the leukocyte lysate remained significantly higher compared to vehicle. In fact, the increase in secreted aSMase (in plasma) has been associated with a consequent decrease in lysosomal aSMase (98). However, similar to the ko model, levels of ceramide were significantly higher at baseline (and following sepsis) as compared to vehicle-treated animals. Similar to other pharmacological inhibitors, desipramine functions as a non-specific inhibitor in the lysosomes. Therefore, it also deactivates other enzymes including the decreased expression of acid ceramidase that produces sphingosine-1-phosphate from ceramide. This could explain the higher levels of ceramide at baseline as well as following sepsis induction in this group as compared to vehicle (96, 114, 115).

To investigate its possible beneficial role in the sepsis continuum, we measured a variety of parameters of host response following pretreatment with desipramine. We observed major differences between the inhibition group (wt animals pretreated with desipramine) and the vehicle wt animals that favor an overall better outcome. However, it is important to note that the present study focused on and addressed the effects of pretreatment with desipramine on sepsis which is not applicable to the clinical situation. Further experiments for the evaluation of the efficacy of desipramine when applied at the time point of sepsis induction as well as several days later is of great clinical value and is required to complete the scientific picture.

Unlike the complete loss of function model (85), desipramine pretreatment and the alleviated aSMase levels did not hinder the intracellular elimination process of bacteria by leukocytes. In fact, treatment of CF mice with pharmacological inhibitors appears to protect against *Pseudomonas aeruginosa* infections (65). Subsequent clinical trials have demonstrated a decrease in the incidence of upper respiratory tract infection and an improvement in overall lung function (116). Here we measured lower CFU in the liver and blood in the inhibition group as compared to vehicle without reaching significance. However, this was not unexpected as desipramine does not retain a short-term antibiotic effect.

A distinct leukocyte phenotype in the inhibition group was also identified and associated with improved parameters of host response. An upregulation in the expression of *Tnfa* and *Il6,* together with higher baseline levels of ceramide, could explain the significantly increased levels of cytokines at the early phase of host response in the inhibition group. However, unlike the continuous increase observed in vehicle animals, systemic cytokine levels significantly dropped in the late phase of host response with abrogated levels of TNF-α (levels reaching baseline) that resulted in attenuated TNF-α dependent IL-6 release. These pharmacological inhibitors are known to have anti-inflammatory properties inhibiting LPS-mediated cytokine release by macrophages. In an endotoxemia shock model, pharmacological inhibition of aSMase also resulted in diminished TNF-α release and improved survival (61, 117).

Following sepsis, a different expression profile of oxidative and stress-associated genes was also observed in desipramine pretreated animals and was associated with abrogated ROS production solely in these animals. Unlike ko animals, the high levels of TNF-α (measured in the inhibition group as well) did not compensate for the impotence in rapid ceramide generation (85). Desipramine pretreatment repressed the expression of *Cyba* and *Ncf2,* which were upregulated in both genotypes following the septic insult, thus abrogating ROS production. In fact, LPS-induced ROS production has been associated with an upregulation in *Cyba*, *Ncf2 and Cybb* (*p22phox, p67phox, gp91phox*) (118). Furthermore, treatment of human endothelial cells with desipramine also repressed TNF-α dependent ROS production (119).

It is important to note however, that the qPCR experiments included only a low number of animals (two per group per time point). Although we could observe

significant differences, this only refers to biological significance. Due to the low number of animals, a statistical analysis of the differences between the groups was not performed. However, we could still observe clear differences in the fold change between the groups following the septic insult (as well as at baseline).

Similar to ko animals, desipramine pretreatment also resulted in the downregulated expression of surface markers CD49d and CD62L on circulating leukocytes following sepsis induction. As previously discussed, these markers are crucial for the leukocytes' rolling and sticking phenomenon which results in leukocyte sequestration and impairment of hepatic perfusion and microcirculation ultimately resulting in organ dysfunction (109, 120, 121). LPS-induced impairment in hepatic microperfusion has been reported following platelet-leukocyte and leukocyte-endothelium interaction. Rolling and sticking leukocytes release cytotoxic mediators such as superoxide, arachidonic acid metabolites and proteases resulting in hypoxia (121). Desipramine pretreatment however resulted in the downregulation of these surface markers subsequently maintaining an unchanged number of rolling and sticking leukocytes following sepsis induction, a parameter that is promising with respect to outcome.

Continuous platelet and leukocyte recruitment through rolling and sticking eventually results in the development of leucopenia and thrombocytopenia; hallmarks in sepsis development (121). Indeed, an increase in rolling and sticking in vehicle wt animals was reflected by a significant drop in leukocyte and platelet counts following the septic insult. Reports indicate that thrombocytopenia increases mortality in ICU sepsis patients where platelet activation and consumption contributes to the development of Disseminated Intravascular Coagulation (DIC) and could lead to the microvessel occlusion that is associated with the development of organ failure (122, 123). Interestingly, following sepsis induction, both leukocyte and platelet counts remained stable in the inhibition group, also promising with respect to outcome.

The overall effect of these altered parameters can be highlighted in the reflection on organ function and survival. Unlike vehicle-treated animals, markers of liver (dys)function and cholestasis (T-Bil and ALT) remained stable in the inhibition group following the septic insult. T-Bil significantly increased solely in the vehicle-treated animals six hours following the septic insult. Similarly, we measured significantly

higher levels of ALT in the vehicle-treated animals in early sepsis compared to inhibition. Another trend was observed in the levels of GGT which started to increase in early sepsis solely in vehicle treated animals without reaching significance.

The summation of the effects of desipramine pretreatment reflected positively on organ function and more importantly on survival. Desipramine pretreated animals registered a 42% survival compared to 20% in both genotypes (wt and ko). Additionally, since desipramine is a non-specific inhibitor of aSMase, it could be argued that the more favorable outcome is unrelated to inhibition of aSMase but is rather a consequence of other effects brought on by the use of the inhibitor. For this purpose, we induced sepsis in ko animals pretreated with desipramine under the same conditions. Interestingly, this did not improve survival and these animals registered a rough 25% survival which proved that the improvement in outcome with the use of the inhibitor is in fact associated with inhibition of aSMase. However, it is still unclear whether the increase in aSMase activity is the course or the consequence with respect to tissue damage and the development of organ failure.

Currently, treatment of sepsis comprises of established stratagems and uniform guidelines for supportive and complement therapy put forward by the health care societies such as the Surviving Sepsis Campaign (SSC) launched by different scientific societies to manage the disease (79, 124, 125). Some of these measures include regimens for antimicrobial therapy, adequate hemodynamic resuscitation and surgical drainage of infected fluid collections (79). Yet, an efficient treatment of the host response is still needed.

During the course of clinical research in sepsis, various elements are critical and required during the investigation, several of which are fulfilled in the present study. These include the need for a well-established and standardized model, an efficient drug as well as patient data. Here, the use of FIASMAs (*i.e.* desipramine) in a clinically translation model of sepsis appears to be promising with respect to treatment and in improving survival through the inhibition of the aSMase enzyme which has been shown to increase in patients throughout the development of the disease (46, 126). Additionally, since FIASMAs are used in the ICU for the treatment of hospitalization-associated depression, our preliminary data through retrospective

data analysis revealed improvement in markers of organ (dys)function with the use of these drugs.

IV- Conclusion

The present study demonstrates a dual role of aSMase in host response. Although the enzyme is crucial in the early phase of host response with respect to the elimination of microorganisms, it is evident that its continuous increase has detrimental effects with poor outcome. The rise in the incidence of sepsis and the limited treatment options highlight the need for effective therapy. In the present study, the use of desipramine presents with promising results with respect to its possible therapeutic use in sepsis and further encourages clinical studies with the use of pharmacological inhibitors of aSMase in well defined septic patients.

V- Summary

The increase in acid sphingomyelinase (aSMase) activity has been implicated in the severity of disease and its fatal outcome. In sepsis, aSMase activity has been shown to continuously increase throughout the continuum from SIRS (systemic inflammatory response syndrome) to severe sepsis. The inhibition of this enzyme with desipramine, a low molecular weight inhibitor, presents promising results in cystic fibrosis patients with improvement in lung function and patient status. We therefore addressed the question of whether secreted aSMase is involved in host response to infection, which is reflected in humoral and functional parameters of immune activation. We identified a dual function of this enzyme in host response where the activity of aSMase is essential during the early phase of host response with respect to bacterial elimination, yet the continuous increase in aSMase activity and ceramide generation presents with detrimental effects in the late phase of host response. We found a different cytokine profile between the genotypes (wt and ko) and yet more striking results following pharmacological inhibition with desipramine (*i.e.* Inhibition group). We observed a hyperresponsive state in the ko animals highlighted by an overwhelming bacterial burden and inflammatory response with poor outcome and presented with a 20% survival, similar to wt. We observed a more favorable overall profile in the inhibition group highlighted by abrogated and alleviated cytokine levels and ROS release associated with a repressed gene expression profile. Additionally, desipramine pretreatment resulted in the downregulation in expression of surface markers with subsequent unchanged values in numbers of rolling and sticking leukocytes. We also measured stability in markers of liver dysfunction in desipramine pretreated animals compared to the other two groups. In summation, the altered parameters reflected on survival with a more favorable survival of 42% in the inhibition group compared to a 20% survival in wt and ko animals. The use of the functional inhibitor desipramine reveals to be promising with respect to treatment of the host response. As it is already FDA approved for the treatment of several human conditions, the present study encourages further research into the use of desipramine for the treatment of host response. The presented data also encourage a clinical study with the use of desipramine in well defined sepsis patients.

Zusammenfassung

Hintergrund und Fragestellung Sphingolipide fungieren neben ihrer vermeintlichen Hauptaufgabe als inerte Strukturkomponenten in Zellmembranen auch als hochaktive Metabolite und Mediatoren bei zahlreichen Stoffwechselprozessen wie zelluläre Stressantwort, Inflammation und Apoptose. Die Metabolite der Sphingomyelin-hydrolyse, insbesondere Ceramid, einschließlich der Aktivität des Schrittmacher-enzyms der raschen Ceramidbildung - die saure Sphingomyelinase, aSMase - sind bei Patienten in der Plasma-sezernierten Form mit Sepsis schweregradabhängig erhöht. Innerhalb der Zellmembran neigt Ceramid zur Selbstaggregation in Form von Makrodomänen, in welchen Rezeptorproteine zu funktionell aktiven Komplexen assemblieren und eine Vielzahl von Signalkaskaden (TNF-Rezeptor, CD95, TLR4 u.a.) in immunkompetenten Zellen verstärken. Die Sekretion der aSMase wird durch pro-inflammatorische Zytokine stimuliert, andererseits vermindern funktionelle Inhibitoren des Enzyms wie Desipramin die Endotoxin-induzierte Zytokinfreisetzung *in-vitro*. Darüber hinaus verbesserte Desipramin in einer klinischen Studie bei cystischer Fibrose sowohl den pulmonalen Infektionsstatus (verbesserte Clearance *P. aeruginosa*) als auch Lungenfunktionsparameter. Vor diesem Hintergrund sollte eine mögliche Rolle des Enzyms und die Effekte einer Inhibition der aSMase bei der Wirtsantwort und bei der Entwicklung eines Organversagens untersucht werden.

Methoden Nach Genehmigung wurden Mäuse einer polymikrobiellen Infektion mittels intraperitonelaer Applikation einer Stuhlsuspension unterzogen. Neben der Überlebensrate wurden die Enzymaktivität, die Ceramidbildbung in zirkulierenden Leukozyten, das Zytokinprofil, Bildung reaktiver Sauerstoffradikale, Organfunktions-parameter, Phagozytose, Bakterienlast sowie zum einen mittels Intravitalmikroskopie die Leukozyten-Endothel-Interaktion, zum anderen mittels Durchflusszytometrie der Phänotyp der zirkulierenden Leukozyten analysiert. Dabei wurden folgende Versuchs-gruppen verglichen: (i) Mäuse mit genetischer aSMase-Defizienz, (ii) deren wt-*littermates*, sowie (iii) Mäuse nach pharmakologischer Inhibition der aSMase mit Desipramin. In Vorarbeiten wurde die Leukozyten-Endothel-Interaktion im (post)sinusoidalen Gefäßbett der Leber charakterisiert.

Ergebnisse Nach Induktion der polymikrobiellen Sepsis entwickelten alle Tiere unabhängig von Genotyp oder Vorbehandlung das erwartete Krankheitsbild. Inhibition verhinderte den Sepsis-induzierten aSMase-Aktivitätsanstieg. Eine veränderte Wirtsantwort spiegelt sich in einer signifikant höheren Bakterienlast im

Blut und in Leberwebe genetisch defizienter Tiere wider, was mit einer signifikant erhöhten Phagozytoseleistung vergesellschaftet ist. Plasma-zirkulierende Zytokinkonzentrationen erlaubten eine Gruppen-bezogene Differenzierung, dabei resultiert die pharmakologische Hemmung in einer veränderten Kinetik und Dynamik der Zytokinfreisetzung. Die Freisetzung reaktiver Sauerstoffradikale war bei pharmakologischer Inhibition vermindert und mit einem korrespondierenden Genexpressionsmuster in zirkulierenden Leukozyten assoziiert. Mittels intravitaler Mikroskopie wurden Unterschiede hinsichtlich der Leukozyten-Endothel-Interaktion in der Leber analysiert: Sechs Stunden nach Sepsisinduktion in wt-Tieren steigt der Anteil rollender Leukozyten von 6 auf 11/100 Leukozyten an, wobei in beiden Vergleichsgruppen bei ähnlichem Ausgangsniveau kein Anstieg zu verzeichnen ist. Ebenso steigt nur in wt die Anzahl adhärenter Leukozyten signifikant an. Ebenso fällt bei aSMase-Defizienz oder Inhibition die Expression des leukozytären Aktivierungsmarker CD49d signifikant vom Ausgangswert ab, was mit der verminderten endothelialen Adhärenz korrespondiert. Bei der Betrachtung von Parametern der Leberschädigung waren lediglich in den genetisch defizienten Tieren diese signifikant erhöht. In der Überlebensanalyse spiegelte sich die überschießende Wirtsantwort der ko-Tiere in einem früheren Versterben im Vergleich zur wt-Gruppe wider (jeweils 20%). Darüber hinaus war die Absterbekinetik in den Inhibitor-behandelten Tieren verzögert und die Überlebensrate erhöht (42%).

Interpretation Die Daten belegen eine wichtige Rolle der aSMase während der Wirtsreaktion bei polymikrobieller Infektion. Dabei kann dem Enzym möglicherweise eine duale, phasenabhängige Rolle zugeschrieben werden: In der frühen Phase ist ein Aktivitätsanstieg essentiell für adäquate Keimelimination und Stressantwort, wobei im *loss-of-function* Modell überraschenderweise eine Hyperreaktivität bei der Wirtsantwort auffällig ist. Bei pharmakologischer Inhibition scheint die Restaktivität (ca. 40% des Basalwerts) für die primäre Keimabwehr ausreichend, für die Hemmung der überschießenden Aktivität jedoch protektiv bezüglich der Stressantwort infektortferner Organe wirksam zu sein. Insgesamt erscheint die überschießende Wirtsantwort positiv beeinflusst. In Zusammenschau mit dem Zytokinprofil, dem verminderten Gewebeschaden, und dem resultierenden Überlebensvorteil sollte ein möglicher positiver Effekt der Inhibition mit einem in anderer Indikation zugelassenen Wirkstoff - wie beispielsweise Desipramin - zeitversetzt nach Infektion überprüft werden.

VI- Abbreviations

ALT	alanine transaminase
APC	Activated Protein C
aSMase	Acid Sphingomyelinase
BW	Body Weight
Cer	Ceramide
CF	Cystic Fibrosis
CFDA-SE	Carboxyfluorescein diacetate succinimidyl ester
CFU	Colony Forming Units
CLP	Cecal Ligation and Puncture
DAMPs	Damage associated molecular patterns
ES	Endothelium Surface
FADD	FAS-associated death domain protein
FDA	Food and Drug Administration
FIASMAs	Functional inhibitors of acid sphingomyelinase
GGT	gamma glutamyl-transferase
HMGB-1	High mobility group protein B1
ICAM-1	Intracellular adhesion molecular-1
ICU	Intensive Care Unit
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-17	Interleukin-17
IP	Intra-peritoneal
IRS-1	Insulin Receptor Substrate 1
IVM	Intravital Microscopy
ko	knock-out
ko-Inh	knock-out plus inhibition
LPS	Lipopolysaccharide
LS	Liver surface
MFI	Mean Fluorescent Intensity
MOF	Multiple Organ Failure
PAMPs	Pathogen associated molecular patterns
PCI	Peritoneal Contamination and Infection
PRR	Pathogen Recognition Receptors
ROS	Reactive Oxygen Species
SIRS	Systemic Inflammatory Response Syndrome
Spp.	Species
SSC	Surviving Sepsis Campaign
T-Bil	Total Bilirubin
TNF-α	Tumor Necrosis Factor-alpha
TNFR	Tumor Necrosis Factor receptor
UK	United Kingdom
US	United States
VCAM-1	Vascular Cell Adhesion Molecule-1
wt	wild type

VII- References

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VIII- Appendix

Declaration of Honor

I, Nayla Jbeily, hereby declare that I have read and understood the course of examination for a doctoral candidate at the Faculty of Biology and Pharmacy of the Friedrich-Schiller University Jena.

I also confirm that I personally prepared and wrote the present dissertation and carried out myself the activities involved in it. The support provided during the work, including significant assistance from my supervisors and coworkers has been indicated in full.

All the sources are acknowledged by means of complete referencing.

I declare that I did not enlist any assistance of a doctoral consult and that no third parties have received either direct or indirect monetary benefits from me for work related to this submitted dissertation.

I declare that the academic work has not been submitted to any other examination authority and that I did not submit the same, a substantially similar or a different dissertation to another postsecondary school.

I am aware that a false declaration will have legal consequences.

Curriculum Vitae

PERSONAL INFORMATION

Name: Nayla Jbeily

Nationality: Lebanese

Date of birth: 24-11-1983

Gender: Female

Marital Status: Single

Children: None

Address: Okenstraße 6, 07745 Jena, Germany

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Skills:

- Experience in Animal handling and experimentation – Felasa B
- Phlebotomy
- Research skills
- Red Cross
- Computer Skills (Microsoft excel, Microsoft PowerPoint, Microsoft word, Sigma plot, Origin, Endnote and others)

Hobbies and Interests:

- Reading
- Sports
- Travelling
- Music
-

EDUCATION

Lebanese Baccalaureate in Life Sciences
Greater Beirut Evangelical School 1986 – 2001

Bachelor of Sciences in Medical Laboratory Technology
University of Balamand 2001 – 2004
Master Degree in Clinical Laboratory, specialization Microbiology
University of Balamand 2006 – 2009

WORK EXPERIENCE

2002-2004 Saint-George Hospital – Achrafieh , Lebanon

- Training in Medical Laboratory Technology in all the sections of the laboratory

2005 – 2009 .Serhal Hospital – Rabieh, Lebanon

- Medical Laboratory Technologist
- Working day and night shifts in all sections of the Laboratory which include Hematology, Microbiology, Urine analysis, Biochemistry, Serology, Blood Banking and Phlebotomy

2008 – 2009 Laboratory Assistant (teaching)

University of Balamand – Faculty of Health Sciences

- Teaching students the different techniques of the micro-biology/bacteriology laboratory – application and analysis

2010-2013 (at present) Research project leader

International Leibniz Research School – University hospital Jena: Experimental Anesthesiology and Intensive Care medicine.

- PhD student

LINGUISTIC PROFICIENCY

Languages	Speaking	Understanding	Writing	Reading
Arabic	Excellent	Excellent	Excellent	Excellent
English	Excellent	Excellent	Excellent	Excellent
German	Good	Good	Good	Good
French	Good	Good	Good	Good

Involvement in Research Studies

I was involved in three research studies:

- Imipenem Resistant *Acinetobacter baumannii* in two Lebanese hospitals: Relatedness and Mechanisms of Resistance (2007-2009 – Publication in process)

- Antimicrobial Susceptibility Patterns of *Haemophilus influenza* and *Streptococcus pneumoniae* in a General University Hospital in Beirut between 2000 and 2004
- Country-wide Spread of Community- and Hospital- acquired Extended Spectrum-beta- Lactamase (CTX-M-15)-Producing Enterobacteriaceae in Lebanon

Scientific Projects Attended

2007	Resistance Trends and $bla_{CTX\text{-}M\text{-}15}$ Gene Transferability in *Salmonella Species* Isolated from Lebanese Patients
2009	Antibiotic Resistance: Overview and Selected Mechanisms
2009	Antibacterial Activity of the Extracts obtained from selected Lebanese plants on MDR Clinical Isolates of *Escherichia coli* and *Klebsiella pneumoniae*
2009	Imipenem Resistant *Acinetobacter baumannii* in two Lebanese Hospitals: Mechanisms of Resistance and Antibiotic Typing Schemes

Scientific Seminars and Conferences

2003	The VII Lebanese National Conference on Infectious Diseases and Clinical Microbiology
2003	The XIX annual medical conference on adolescent mental health, children's rights and services
2003	Seminar on Human rights organized by the "Arab Institute for Human Rights"
2007	Antibiotics: From Theory to Practice
2011	Sphingolipids – Signals and disease (17-18 February)
2011	Weimar Sepsis Update (7-10 September)
2012	Wissenschaftliche Arbeitstage der Deutschen Gesellschaft für Anästhesiologie und Intensivmedizin (10-11 February)
2012	Annual Conference of the Association for General and Applied Microbiology (VAAM) (18-21 March)
2012	Seventh International Shock Congress – 35th Annual Conference on Shock 9-13 June)
2012	International Meeting on Antimicrobial Peptides (30-31 August)

Publications

- **Jbeily N,** Suckert I, Gonnert FA, Acht B, Bockmeyer CL, *et al.* (2013). Hyperresponsiveness of mice deficient in plasma-secreted sphingomyelinase reveals its pivotal role in early phase of host response. *J. Lipid Res*, 54(2):410-24
- Gonnert F, Rechnagel P, Madlen S, **Jbeily N**, Dahlke K, *et al.* (2011). Characteristics of Clinical Sepsis in a Reliable and Reproducible Rodent Sepsis Model. *Journal of Surgical Research*, 170 (1), 123-134.
- Recknagel P, Gonnert FA, Halilbasic E, Gajda M, **Jbeily N**, *et al.* (2013). Mechanisms and functional consequences of liver failure substantially differ between endotoxemia and fecal peritonitis in rats. *Journal of Liver International* 33(2):283-93.
- **Jbeily N**, Grossmann SD, Suckert I, Gonnert FA, Ludwig T, *et al.* (2013). Identification of a distinctive leukocyte phenotype following pharmacological inhibition of aSMase during host response (**submitted** to the Disease *Models and Mechanisms* Journal).

References

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- PD Dr. rer. nat./med. habil Ralf A. Claus: Head AG Molecular Mechanisms of Organ Failure/Exp. Anesthesiology, Jena University Hospital, Center for Sepsis Control and Care (CSCC), Clinic for Anesthesiology and Intensive Care – Jena, Germany – 0049.(0) 3641.9.325860
- Dr. Ziad Daoud: Asscociate Professor, Clinical Microbiology, Department of Biomedical Sciences, Faculty of Medicine and Medical Sciences, University of Balamand, Lebanon – 00961.6.930279 ext: 3819
- Dr. Raffael Zarilli: Dipartimento di Scienze Mediche Preventive, Università di Napoli Federico II, Via Pansini 5, 80131 Napoli, Italy. Phone: 0039-081-7463026.

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Nayla Jbeily 14-03-2013

List of Publications:

- **Jbeily N**, Suckert I, Gonnert FA, Acht B, Bockmeyer CL, *et al.* (2013). Hyperresponsiveness of mice deficient in plasma-secreted sphingomyelinase reveals its pivotal role in early phase of host response. *J. Lipid Res,* 54(2):410-24.
- Gonnert F, Rechnagel P, Madlen S, **Jbeily N**, Dahlke K, *et al.* (2011). Characteristics of Clinical Sepsis in a Reliable and Reproducible Rodent Sepsis Model. *Journal of Surgical Research*, 170 (1), 123-134.
- Recknagel P, Gonnert FA, Halilbasic E, Gajda M, **Jbeily N**, *et al.* (2013). Mechanisms and functional consequences of liver failure substantially differ between endotoxemia and fecal peritonitis in rats. *Journal of Liver International,* 33(2):283-93.
- **Jbeily N**, Grossmann SD, Suckert I, Gonnert FA, Ludwig T, *et al.* (2013). Identification of a distinctive leukocyte phenotype following pharmacological inhibition of aSMase during host response (**submitted** to the *Disease Models and Mechanisms Journal*).
- **Jbeily N,** Claus RA, Bauer M, Gonnert FA. Comparison of Carboxyfluorescein diacetate succinmidyl ester (CFSE) and Rhodamine 6G for *in-vivo* labeling of leukocytes (**In Preparation**).

List of Scientific Conferences:

2011 Sphingolipids – Signals and disease (17-18 February) Essen, Germany [Presentation – **Talk**].

Update of the project was presented again by a **Poster** presentation in Sphingolipids – Signals and disease (October 2012) [substituted by Ha-Yeun Chung because of inaccessibility due conflict between my nationality and the location of the conference].

2011 Weimar Sepsis Update (7-10 September) Weimar, Germany [Presentation – **Talk**].

2012 Wissenschaftliche Arbeitstage der Deutschen Gesellschaft für Anästhesiologie und Intensivmedizin (10-11 February) Würzburg, Germany [Presentation – **Talk**].

2012 Annual Conference of the Association for General and Applied Microbiology (VAAM) (18-21 March) Tübingen, Germany [**Attended**].

2012 Seventh International Shock Congress – 35th Annual Conference on Shock (9-13 June) – Miami, Florida, USA [Presentation – **Poster**]

2012 International Meeting on Antimicrobial Peptides (30-31 August) Leipzig, Germany [**Attended**].

Additional Training and Activities

- Training in Adobe Photoshop
- Training is Scientific presentations
- Intravital Microscopy training supervised by Prof. Bauer and Dr. Gonnert
- FELASA B: Animals Handling and Surgery
- Summer school on Clinical Biophotonics
- Workshop on Fungal Infections and Innate Immune Response
- Additional expert training in Microbiology supervised by Prof. Rödel
- Supervision of several projects of Bachelor, Master and Medical students

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Finally, I am deeply grateful to God for all His blessings and for my parents, siblings and friends whose support and help encouraged and allowed me to follow my goals and dreams.